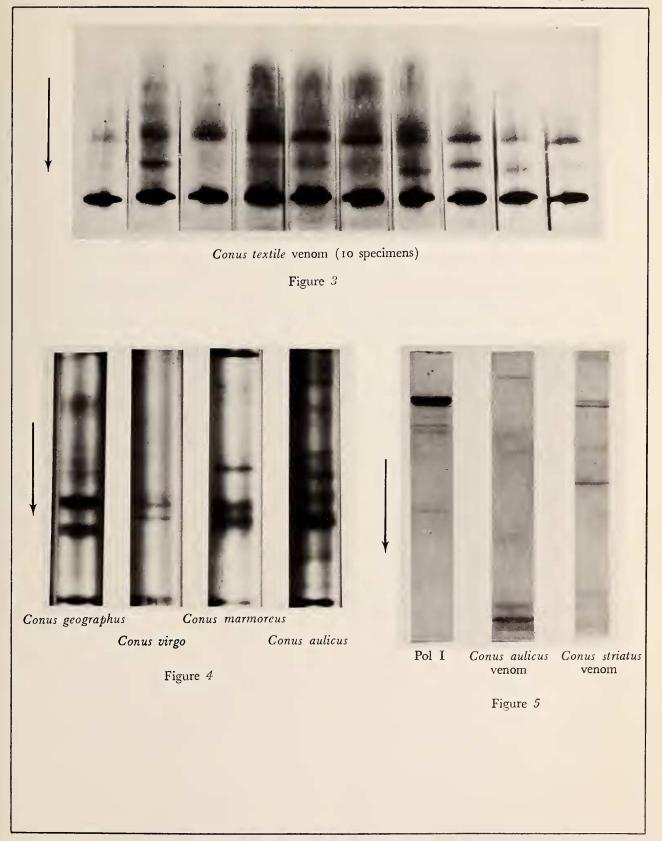
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Test for Protein Nature of Toxin from Conus textile venom

Tubes 1 and 2 were incubated at 37°C for 2 hrs then treated with 1 mg pronase and incubated for another 2 hrs. Tube 3 was incubated once at 37°C for a total of 4 hrs. All tubes were dialyzed against 500 ml of normal saline solution (0.15 NaCl) for 3 hrs, then overnight and for another 2 hrs the following day. A fifth tube containing 600 μ l pooled venom was left over ice during dialysis of the other 4 tubes. All samples were injected intracisternally.

A. (Incubation Protocol)							
Sample	Volume 20% venom (µl)	Volume 0.15 M NaCl (µl)	Wt Pronase	Volume 0.1 M NH₄HCO₃ (µl)			
1	600	_	2.0 mg	100			
2		600	2.0 mg	100			
3	600	_	_	100			
4	600	_	_	_			

B. (Results of Injection)								
Sample injected	Wt. of mouse grams	Volume injected (µl)	Observa	ations				
1. Pronase digested venom	28.4	50	all had labored breathing	but recovered after				
	31.0	50	2 min; shallow abdomina	breathing, scratching				
	32.8	50	of sides; moving about in circles; shivering;					
	26.9	50	found dead the following	morning.				
	27.9	50						
2. Pronase control	28.4	50	found dead the following	morning				
	30.1	50	died after 2 days	0				
	26.6	50	died on the following day					
	27.6	50	and on the following day					
3. Incubated venom (undigested)	34.6	50	died after 3 min	gasped for air;				
(unalgested)	37.5	50	died after 4 min, 55 sec.	convulsions &				
	35.2	50	died after 1 min, 5 sec.	shivering;				
	29.2	50	died after 2 min	tremors of forelimbs				
4. Dialyzed venom	28.0	50	died after 2 min, 55 sec.	abdominal-mouth breathing				
	35.2	50	found dead after 2 days	bloodshot eyes; blood clots on hind limbs and tail				
	25.5	50	found dead the following	day				
	33.9	50	died after 2 min, 4 sec.	twitching; abdomin-				
	32.5	50	died after 54 sec.	al-mouth breathing;				
	24.7	50	died after 2 min, 10 sec.	convulsions before death				
5. Untreated venom	27.1	50	died after 2 min, 54 sec.	violent convulsions;				
	35.7	$<\!50$	died after 3 min, 4 sec.	twitching of hind				
	25.4	$<\!50$	died after 2 min, 19 sec.	legs; abdominal 7				
	34.1	50	died after 1 min, 30 sec.	mouth breathing				

		A. (Incub	ation Protocol)			
Tube #	Volume 20% venom (µl)	Volume 0.15 M NaCl (µl)	Volume Pronase (µl)	Volume 0.01 M NH4HCO (µl)	Volume 3 0.1 M NH4HCO3 (μl)	
1 2	50 50	50 50	10 —	10	20 20	
		B. (Resul	ts of Injection)			
Sample injected		Wt. of mouse grams	Volume injected (µl)	Obse	ervations	
1. Pronase digested venom	L	15.7 15.8 17.2	50 50 80	all behaved normally; no sign of weakness		
2. Undigested venom		16.6 13.8 12.7	50 50 60	died after 144 min died after 114.5 min died after 107 min	all had spasms after 1 hr & 40 min; rolled around; slow labored breathing & gasping before death	

Protein Nature of Toxin from Conus tulipa Venom

Tubes were incubated at 37° C for 2 hrs, dialyzed against 500 ml normal saline solution (0.15 M NaCl) overnight, then again for 4 hrs. 100 λ of 0.15 M NaCl was added to the dialyzed preparations immediately before injection (intraperitoneal). The pronase solution used contained 30 mg of pronase per ml of 0.01 M NH₄HCO₃.

effects of *Conus geographus*, *C. textile* and *C. magus* are distinct from each other. What is the molecular basis for the different biological effects? In addition, possible enzymatic activities of some of the other proteins present in these venoms can also be examined more rigorously. Venoms have been sources of purified enzymes that have become standard reagents in biochemistry. The *Conus* venoms, with several hundred species in the genus, are clearly a rich potential source for such enzymes.

ACKNOWLEDGMENTS

This work was supported by the National Research Council of the Philippines (Research Grant I. D. -25). Eric Flores and Vicente Castillo participated in some of the initial experiments. We are grateful to Mrs. Gerarda Abanil, Mr. Evaristo Zambo and Mrs. Mildred Simolde for sending us *Conus* specimens. Dr. Nelia Cortes-Maramba gave us valuable advice regarding bioassays. Mario Santos took the photographs, and Prof. Bienvenido Miranda loaned us photographic equipment. Without funds from the China Medical Board, the gel electrophoresis experiments would not have been possible.

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Censuses of Rocky Shore Prosobranchs from Washington and Costa Rica

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(1 Text figure)

INTRODUCTION

MOST TROPICAL COMMUNITIES harbor more kinds of organisms than do comparable temperate communities. The zone between the tidemarks is no exception. Many more kinds of snails can be found on beaches of the tropical Eastern Pacific than on more northern ones. If more kinds of snails live together on tropical beaches, then the individual snails must use the resources of the beach in a different manner than do their counterparts on the temperate beach.

Most species occupy only a few of the available habitats in an area, and a species' distribution can generally be characterized fairly precisely with a short list of habitats. Organisms that live on rocky shores are subjected to repeated physical stresses, and these stresses are generally reflected in the distributions of the organisms. Exposure to air is the most obvious stress, and diversity generally decreases markedly at upper shore levels where exposures are long and frequent (JOHNSON, 1970). Wave action is also stressful and community composition generally changes along gradients of wave intensity (*e. g.*, RICKETTS & CALVIN, 1968).

To an extent an organism can escape the stresses of wave action or air exposure by selecting a favorable microhabitat. Rocky shores are formed by 2 major habitat types, (1) rock reefs, continuous expanses of rock interspersed with various kinds of crevices which provide hiding places, and (2) cobble areas, stones of various sizes on hard, gravel, or soft substrates which provide hiding places for various motile organisms on the undersurfaces of the stones.

The present study was undertaken to compare habitats of intertidal snails of the Friday Harbor area, Washington, and Playas del Coco, Costa Rica. Snails were chosen because they are relatively well known (KEEN, 1971) and easy to sample quantitatively. To characterize the distributions of the various species, quantitative samples were taken from both latitudes. At each sampling site, shore height, degree of wave exposure, and substrate type were recorded.

METHODS

Costa Rica

Quadrat samples were taken during low tide periods from the Playas del Coco beach (Figure 1) between 8 February 1970 and 21 March 1970 (Samples 1 to 31, Table 1) and between 7 to 14 February 1971 (Samples 32 to 38). I chose some particular habitat, defined by type (rock reef, tidepool, or cobble; cobble quadrats including one or more movable stones from baseball size up, resting on mud, gravel or coral debris), height on the beach, and exposure. Then an area, usually $1 \text{ m} \times 1 \text{ m}$ or $2 \text{ m} \times 2 \text{ m}$ was outlined with a rope. Gastropods and chitons were hand-picked from the quadrat and later sorted and measured in the field laboratory. Data for prosobranchs (amended to include Bulla punctulata, Siphonaria maura, and S. gigas) are presented in this report (Table 2). Representative specimens of each species were preserved, and the remaining individuals were returned to the sampling sites.

Heights of all 1970 quadrats were measured relative to the water level and to a fixed tide gage. Timed readings from this gage, in ft (X), were correlated with interpolated readings for Puntarenas, Costa Rica (Y) from the Pacific Tide Table (ANONYMOUS, 1969):

 $Y = 1.018 X + 1.688; N = 24, r^2 = 0.94, F_{1,22} = 356.89.$ Since heights were not measured in 1971, the 1971 quad-

		Height	Exposure	Nur Prosobra	Density ³	
Number	Class	cm Class		Total	Common ¹	per m ²
		Sai	nplesfrom Rock Habi	ats		
34	F	?	K	4	4	98
25	F	216	W	2	2	158
7	F	186	R	3	3	126
8	D	138	Z	10	9	243
38	D	138	Z	8	8	253
12	С	102	R	6	5	11
11	В	66	Р	7	5	32
37	В	66	Р	8	7	32
10	В	60	Т	8	7	13
15	В	57	Т	9	6	10
14	В	57	Т	7	6	13
32	В	2	Р	9	7	153
35	В	?	I	7	6	82
33 ²	В	?	I	3	1	1100
		S	amplesfrom Tidepoo	ls		
20	E	171	R	10	6	15
28	D	147	Х	8	8	144
29	D	147	Х	9	6	698
			ples from Cobble habi			
26	E	171	Х	3	2	182
4	D	126	R	3	2	1125
22	В	57	W	17	11	190
5	В	54	S	11	5	8.6
1	В	51	Х	14	10	54
2	В	51	Х	14	10	21
21	В	51	Т	12	7	296
9A	В	51	S	8	6	6.6
9B	В	51	S	4	1	1.1
3	В	51	R	10	7	659
19	Α	42	S	15	6	167
6	А	42	Р	9	6	120
18	А	42	Р	7	3	60
16	А	21	Z	25	16	34
17	А	21	Z	21	15	151
13	A	21	Р	4	3	2.5
36	A	?	Р	19	11	16

Characteristics of samples collected at Playas del Coco, Costa Rica

Height and exposure classes, see text.

¹More than one individual in sample.

 ^{2}A 10 \times 10 cm² plot from area of sample 35; data not included in any calculations. Areas sampled: $6m^{2}$ (11, 37), $4m^{2}$ (34, 7, 15, 14, 10, 12, 2, 9A, 9B, 5, 3, 16, 36, 1, 4), $3m^{2}$ (8, 38), $2m^{2}$ (20, 28, 6, 13), $1m^{2}$ (25, 32, 35, 22, 21, 17, 19, 18), $0.5m^{2}$ (26), $0.3m^{2}$ (29).

³The following species were not counted: *Hipponix pilosus* (Samples 13, 14, 15, 16, 17, 36), *Fossarus* sp. (Sample 20), and *Crucibulum umbrella* (Sample 16).

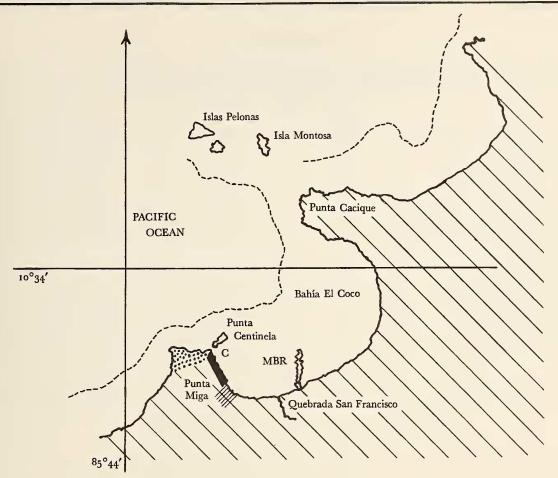


Figure 1

Playas del Coco, Costa Rica, and Vicinity

Dashed line, 18m contour. The various exposure categories are indicated by the following marks on the adjacent land: exposed area, dots; protected area, black; silted area, close hatch. C, area of reef of dead coral. MBR, mid-bay reef

rats had to be assigned height from their locations relative to other quadrats. For this purpose, quadrats were grouped into the height classes: A (< 0.46m), B (0.46 to 0.92 m), C (0.92 to 1.23 m), D (1.23 to 1.54 m), E (1.54 to 1.85 m) and F (> 1.85m). Some samples were taken from sloping or vertical surfaces, and occupied a larger vertical span than a height class. The class assigned included the mean of the upper and lower edges of the quadrat.

All sites were also classified by exposure using an arbitrary scale from P, most exposed, to Z, most protected. P, R and S are on or south of Centinela, and T, W, X and Z run along the southern edge of Bahía El Coco, with Z being heavily silted and experiencing little wave action (Figure 1). Samples 33 and 35 were taken from the exposed side of the offshore island Pelona, and are more exposed than P. Sample 34 was from the landward side of Pelona, and comparable to S or T. The island sites are designated "I" in the exposure column of Table 1.

Washington

Samples were taken from 2 small reefs, Bird Rock and Turn Rock, on opposite sides of Shaw Island, Washington. Two 1 m wide strips were searched from the top of the Bird Rock reef to the water line on 26 July 1968, and

Frequency, density, and sizes of individuals of species collected at Playas del Coco, Costa Rica, 1970 and 1971.

	Freq	uency	N	umbers per n	n²	— Number of	Size
Species	Rock ¹	Cobble	Peak	Mean	SD	Individuals Collected	Range
Diodora inaequalis (Sowerby, 1835)	0	1	0.2	0.2	_	1	29
Fissurella longifissa Sowerby, 1863	4	0	7.0	3.7^{2}	2.9	18	11-25
Fissurella microtrema Sowerby, 1835	1	0	0.2	0.2	_	1	15
Fissurella virescens Sowerby, 1835	8	4	62.0	8.3	17.8	202	16-44
Collisella pediculus (Philippi, 1846)	1	0	7.0	7.0		7	9-17
Notoacmea biradiata (Reeve, 1855)	2	1	17.5	6.8	9.4	44	6-11
Notoacmea fascicularis (Menke, 1851)	2	3	8.0	3.5	3.6	29	4-15
Notoacmea filosa (Carpenter, 1865)	1	7	2.5	2.0	3.3	31	8-21
Scurria mesoleuca (Menke, 1851)	0	1	1.0	1.0	_	1	17
Scurria stipulata (Reeve, 1855)	2	0	1.0	0.6	0.6	2	20-21
Tegula pellisserpentis (Wood, 1828)	0	1	3.7	3.7	_	11	7-23
Tegula panamensis (Philippi, 1849)	0	1	1.0	1.0	_	1	4
Turbo saxosus Wood, 1828	0	1	0.2	0.2	_	1	9
Tricolia phasianella (Philippi, 1849)	2	0	3.3	2.9	0.6	6	3-4
Nerita scabricosta Lamarck, 1822	4	1	89.2	27.0	36.1	447	3-43
Nerita funiculata Menke, 1851	6	9	640.0	91.2	168.1	967	1-13
Littorina aspera Philippi, 1846	3	Ő	132.0	80.7	48.1	573	2-15
Littorina modesta Philippi, 1846	2	0	9.2	9.22	_	37	6-12
Rissoina sp.	0	3	1.5	0.9	0.7	8	4-5
Modulus cerodes (A. Adams, 1851)	0	1	0.2	0.2		1	4
Cerithium adustum Kiener, 1841	0	3	3.0	1.2	1.5	15	9-51
Cerithium stercusmuscarum Valenciennes, 1833	1	1	16.0	9.0	9.9	33	3-21
	0	1	1.0	9.0 1.0		1	6
Triphora sp. Planaxis planicostatus Sowerby, 1825		2	1120.0	433.5		1217	3-17
	1			455.5			5-17
Hipponix pilosus (Deshayes, 1832)	2	4	+		- 10.1	+	1-12
Fossarus sp.	5	0	23.0	8.12	10.1	36	
Calyptraea conica Broderip, 1834	0	1	1.0	1.0	-	1	14
Crepidula aculeata (Gmelin, 1791)	0	1	0.2	0.2	-	1	9
Crucibulum umbrella (Deshayes, 1830)	0	2	0.2	0.2^{2}	_	1	19
Erato scabriuscula Sowerby, 1832	0	2	1.0	0.6	0.6	2	n.m.
Cypraea cervinetta Kiener, 1843	0	1	1.0	1.0	_	1	30
Cymatium vestitum (Hinds, 1844)	0	2	1.0	0.6	0.6	2	14-22
Bursa caelata (Broderip, 1833)	0	5	17.0	4.0	7.3	26	8-23
Muricanthus princeps (Broderip, 1833)	1	1	1.0	0.7	0.3	4	47-85
Muricopsis zeteki Hertlein & Strong, 1951	0	1	1.0	1.0	-	4	6-14
Aspella pyramidalis (Broderip, 1833)	0	2	1.7	1.3	0.5	11	9-18
Thais speciosa (Valenciennes, 1832)	6	7	8.0	1.9^{2}	2.5	45	6-22
Thais biserialis (Blainville, 1832)	4	1	25.3	11.1^{2}	12.4	133	4-38
Thais melones (Duclos, 1832)	9	11	13.3	5.0	3.4	236	6-43
Acanthina brevidentata (Wood, 1828)	9	2	84.0	24.0^{2}	29.4	640	6-26
Purpura pansa Gould, 1853	1	0	0.3	0.3	-	1	32
Morula lugubris (C. B. Adams, 1852)	0	4	4.0	1.9	1.9	10	3-5
Bailya anomala (Hinds, 1844)	0	2	0.2	0.2	0.0	2	n. m .
Cantharus gemmatus (Reeve, 1846)	0	1	5.0	5.0	-	5	9-26
Cantharus sanguinolentus (Duclos, 1833)	0	8	18.0	3.2	6.0	46	6-18
Engina maura (Sowerby, 1832)	0	8	17.0	3.5	5.6	51	6-13
Engina tabogaensis Bartsch, 1931	0	3	3.2	1.6	1.4	16	4-10
Columbella fuscata Sowerby, 1832	0	1	0.2	0.2	_	1	15
Columbella labiosa Sowerby, 1822	1	0	0.7	0.7	_	3	13-20
Columbella major Sowerby, 1832	0	6	39.0	9.0	14.9	94	6-22
Columbella sonsonatensis (Mörch, 1860)	0	1	1.0	1.0	-	1	n. m .
Anachis costellata (Broderip and Sowerby, 1829)	4	0	82.0	25.7	24.6	358	5-18
Anachis fluctuata (Sowerby, 1932)	0	3	4.0	2.3	1.4	16	6-17

Tab	ole 1	2	con	tinı	led	

	Frequ	iency	N	umbers per n	1 ²	- Number of	Size
Species	Rock ¹	Cobble	Peak	Mean	SD	Individuals Collected	Range mm
Anachis nigricans (Sowerby, 1844)	2	5	353.0	67.8	131.1	232	4-8
Anachis lentiginosa (Hinds, 1844)	5	9	80.5	17.8	23.1	493	3-8
Anachis rugulosa (Sowerby, 1844)	3	0	44.6	29.8	25.4	268	3-8
Anachis pardalis (Hinds, 1843)	0	2	1.0	0.8	0.2	4	6-8
Anachis pygmaea (Sowerby, 1832)	5	6	19.0	7.2	7.2	166	2-6
Mitrella delicata (Reeve, 1859)	1	7	60.0	19.5	21.7	182	4-12
Mitrella guttata (Sowerby, 1832)	0	2	10.0	7.0	4.2	44	7-13
Nassarina melanosticta (Pilsbry and Lowe, 1932)	0	1	2.0	2.0	_	2	n.m.
Leucozonia cerata (Wood, 1828)	1	3	1.2	0.5	0.5	9	5-30
Opeatostoma pseudodon (Burrow, 1815)	5	6	14.0	3.0	4.2	62	8-48
Mitra crenata Broderip, 1836	0	1	0.5	0.5		2	7-13
Mitra lens Wood, 1828	0	6	3.0	1.1	1.1	9	5-18
Mitra tristis Broderip, 1836	0	2	1.0	0.6	0.6	2	9-11
Subcancilla attenuata (Broderip, 1836)	0	1	0.2	0.2	_	1	10
Thala solitaria (C. B. Adams, 1852)	0	1	0.5	0.5	_	2	10-13
Conus gladiator Broderip, 1833	0	1	0.2	0.2	_	1	n.m.
Conus purpurascens Sowerby, 1833	1	5	1.0	0.5	0.3	8	9-47
Conus nux Broderip, 1833	3	7	2.7	1.2	1.0	43	5-19
Strictispira stillmani Shasky, 1971	0	1	1.0	1.0	_	1	n.m.
Bulla punctulata A. Adams 1850	0	2	2.0	1.1	1.3	9	8-11
Siphonaria gigas Sowerby, 1825	2	0	1.0	0.6	0.6	5	15-21
Siphonaria maura Sowerby, 1835	7	4	42.0	6.0	12.6	98	8-29
Columbellid juvenile	1	0	0.5	0.5	_	1	5
Anachis No. 4	0	1	1.0	1.0		1	12
Latirus No. 4	0	1	0.2	0.2	_	1	5
Conus juvenile	0	1	1.0	1.0	_	1	6
Total	118	196	1125			6970	

n.m., Not Measured

¹includes Tidepool quadrats.

²Density estimate for one sample omitted from total.

supplemented with samples from tidepool and gravel areas. The single Turn Rock transect, collected 27 July 1968, was also supplemented with samples from tidepool and gravel areas. Individuals were identified and measured in the laboratory, and returned to the islands (Table 3). Quadrat heights were measured and quadrats were classified by height as had been done for the tropical samples (Table 4).

Limpets were not collected from these transects. The several species of limpets include the majority of the individuals on Bird Rock and Turn Rock, and some correction is necessary to make the temperate data comparable with the tropical data. DAYTON (his table 5, 1971) gives densities for limpets on transects he has taken on Turn Rock. Total prosobranch diversities and densities for both islands can be obtained by adding Dayton's limpet densities to the numbers in my own samples. Thus, 5.3 species per sample and 101.7 individuals/m² are adjusted to 9 species per sample and 400 individuals/m².

The Bird Rock and Turn Rock sites are typical of Friday Harbor area sites as described by KOZLOFF (1973). Site descriptions are given by VANCE (1972) and DAYTON (1971).

CHARACTERISTICS OF THE TROPICAL SITE

The intertidal zone at Playas del Coco consists of a narrow band of rock reef interrupted by sandy or silty areas and by areas with gravel, stones, or boulders. In most intertidal areas, the beach slope is fairly shallow. The sand